NEUROSECRETORY CELLS IN GANGLIA OF THE ROACH, BLABERUS CRANIIFER [1]

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Neurosecretory cells have been described in many groups of insects (Scharrer and Scharrer, 1954). Most observations have been made upon brain, subesophageal and frontal ganglia, and corpora cardiaca. There is relatively little information concerning neurosecretion by cells within the thoracic and abdominal ganglia of insects. Day (1940) described neurosecretory cells in the abdominal ganglia of Lepidoptera, and Köpf (1957) found neurosecretory cells in the thoracoabdominal ganglion of *Drosophila*. E. Thomsen (1954) found no evidence that neurosecretory materials were accumulating on either side of ligatures of abdominal connectives in *Calliphora* and concluded that these connectives did not carry neurosecretory products.

During an investigation of the functional roles of the neurosecretory materials accumulated within the corpora cardiaca of the roach, *Blaberus* (Özbas and Hodgson, 1958), the search for appropriate controls, *i.e.*, tissues which would contain only non-secretory neurons, prompted an examination of the thoracic and abdominal ganglia of this species. It immediately became apparent that neurosecretory cells of more than a single type were present in all of these ganglia. Since the existence of neurosecretory cells had not been previously reported within the thoracic or abdominal ganglia of Blattaria, and since this order of insects is commonly utilized in experiments on neurosecretion, it appeared worthwhile to make a detailed study of the neurosecretory cells at these hitherto unstudied loci.

The object of the present report is to describe the types of neurosecretory cells present in the thoracic and abdominal ganglia of *Blaberus*, and in so far as it is possible, to trace the movements of secretions produced by these cells, using evidence from ligation and histological studies. The relationships of the neurosecretory cells within the ganglia to secretory cells located elsewhere within the nervous system are also considered.

Materials and Methods

The last three larval instars and adults of *Blaberus craniifer* were studied. No significant differences correlated with sex or developmental stages were found among specimens of this group. Only adults were used in ligation experiments because their larger size afforded technical advantages for the operations.

Two fixatives and two stains were used on each type of preparation, so that by

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comparing the results of the various methods it was possible to rule out artifacts produced by any one fixative or stain. Either Bouin or Helly fixatives were used. The stains were the chrome hematoxylin phloxin stain of Gomori (1941), hereafter designated CHP, or the aldehyde fuchsin stain (Gomori, 1950) as modified by Halmi (1952) and Dawson (1953), hereafter designated AF. All sections were cut 5 microns in thickness.

Ligatures were prepared from Clark's Size A black nylon thread. A single strand was teased from this thread and tied around one of the two connectives which pass between the ganglia. The operations were performed through incisions in the ventral sides of unanesthetized animals, and the ligature sites were varied in different animals so that blocks of connectives between each of the thoracic and abdominal ganglia were represented in the total series of 44 operated animals. The operated roaches were maintained in separate pint jars and fed dogfood, fruit, and water, this being the same diet given the standard laboratory colony of *Blaberus*. The animals were sacrificed 5 to 40 days after the operation, the nerve cords dissected out, and histological preparations made by the methods outlined above.

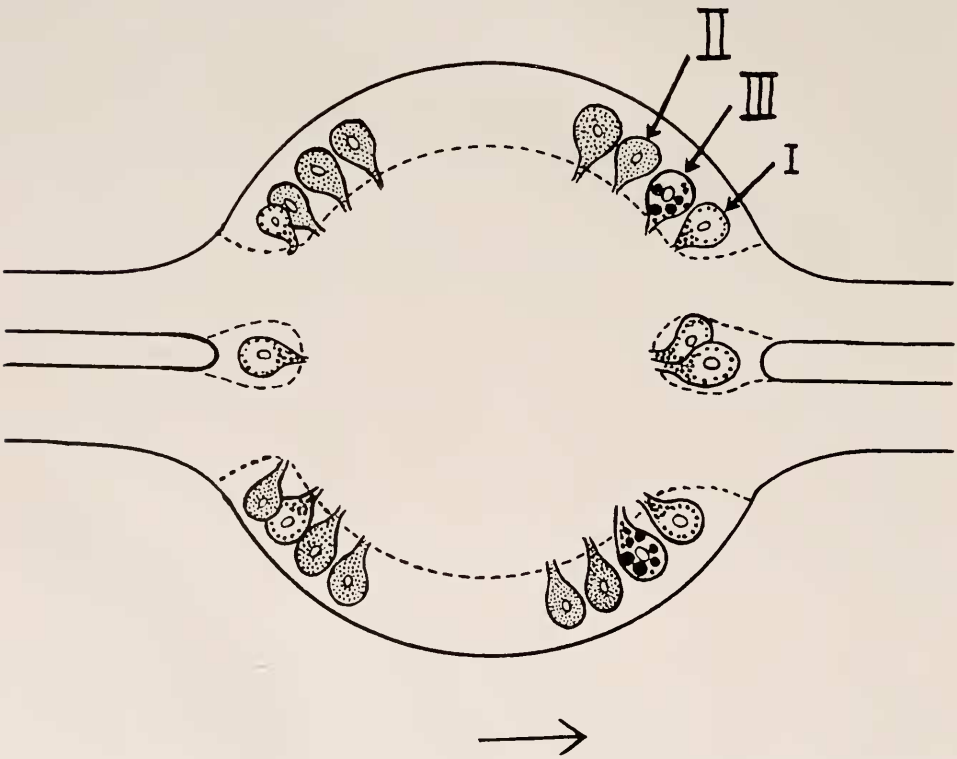

FIGURE 1. Diagrammatic representation of a frontal section through the center of a thoracic ganglion, showing arrangement of neurosecretory cells. The dotted lines separate the peripheral areas which are occupied by cell bodies from the central neuropile mass. Roman numerals designate types of neurosecretory cells—see text. The arrow under the diagram points anteriorly, toward the animal's head.

Results

1. *Neurosecretory cells within the ganglia*

The thoracic and abdominal ganglia were found to contain three types of neurosecretory cells, as shown diagrammatically in Figure 1, and in the photographs A, B, and C of Figure 2. These cells can be differentiated from ordinary neurons by their size, staining characteristics, and the presence of characteristic granules or droplets within their cell bodies and axons. The cell bodies of all three types of neurosecretory cells measure 40 to 60 microns in their longest diameters, 35 to 50 microns in their shortest diameters, and have ellipsoid nuclei measuring 15 to 18 microns in their shortest diameters and 18 to 22 microns in their greatest diameters. The nucleoli are also conspicuous in many of the stained neurosecretory cells. The distinguishing characteristics of the three types of cells are described in the following paragraphs.

Type I (see Fig. 2A). The cell bodies and axon hillocks of these cells contain granules which stain deep purple in AF and dark blue in CHP. There are 5 to 10 such cells in each thoracic and abdominal ganglion, and they are located in the outer portion of the ganglion near the origin of the connectives (see Fig. 1).

Type II (see Fig. 2B). These neurosecretory cells have very small granules distributed uniformly throughout the cell body. The granules stain green with AF and red with CHP. These are the most common neurosecretory cells in thoracic and abdominal ganglia, and there are at least several dozen cells of this type distributed generally throughout the periphery of each ganglion (see Fig. 1).

Type III (see Fig. 2C). Only two neurosecretory cells of this type appear to be located within any one ganglion. They are seen only after Helly fixation and never after use of Bouin solution. The two cells are found in the anterior lateral part of the ganglion, one cell on each side of the ganglion (see Fig. 1). They are characterized by the presence of large droplets which stain orange with AF. These droplets have diameters of 3 to 11 microns. They resemble the "colloid droplets" described by E. Scharrer (1941) within the cells of the preoptic nucleus of the fish, *Fundulus*. There was no indication of cycles of formation or alteration of the droplets in the roach sections such as reported in *Fundulus*.

2. *Observations on normal axons and connectives*

Neurosecretory materials from all three types of neurosecretory cells were observed within axons extending into connectives between ganglia. Small granules, such as found in type I cells, are usually found in no more than one or two axons per section of connective (Fig. 2F). These granules fill the axons, where found, and they appear to move in definite oriented chains.

Secretory material from type II cells is best seen in axons after staining with CHP. This material is the most abundant in axons, and is seen in practically every section of connectives between ganglia. Orange material, presumably from type III cells, was seen in axons of only two animals. The rarity of this material in axons may reasonably be explained by the rarity of the type III cells. When observed in axons, the orange material was not in the form of round droplets, but had the form of elongated rods.

Many glia cells were seen between axons in the connectives and also in the ganglia. These cells were described by B. Scharrer (1939) in the brain, sub-

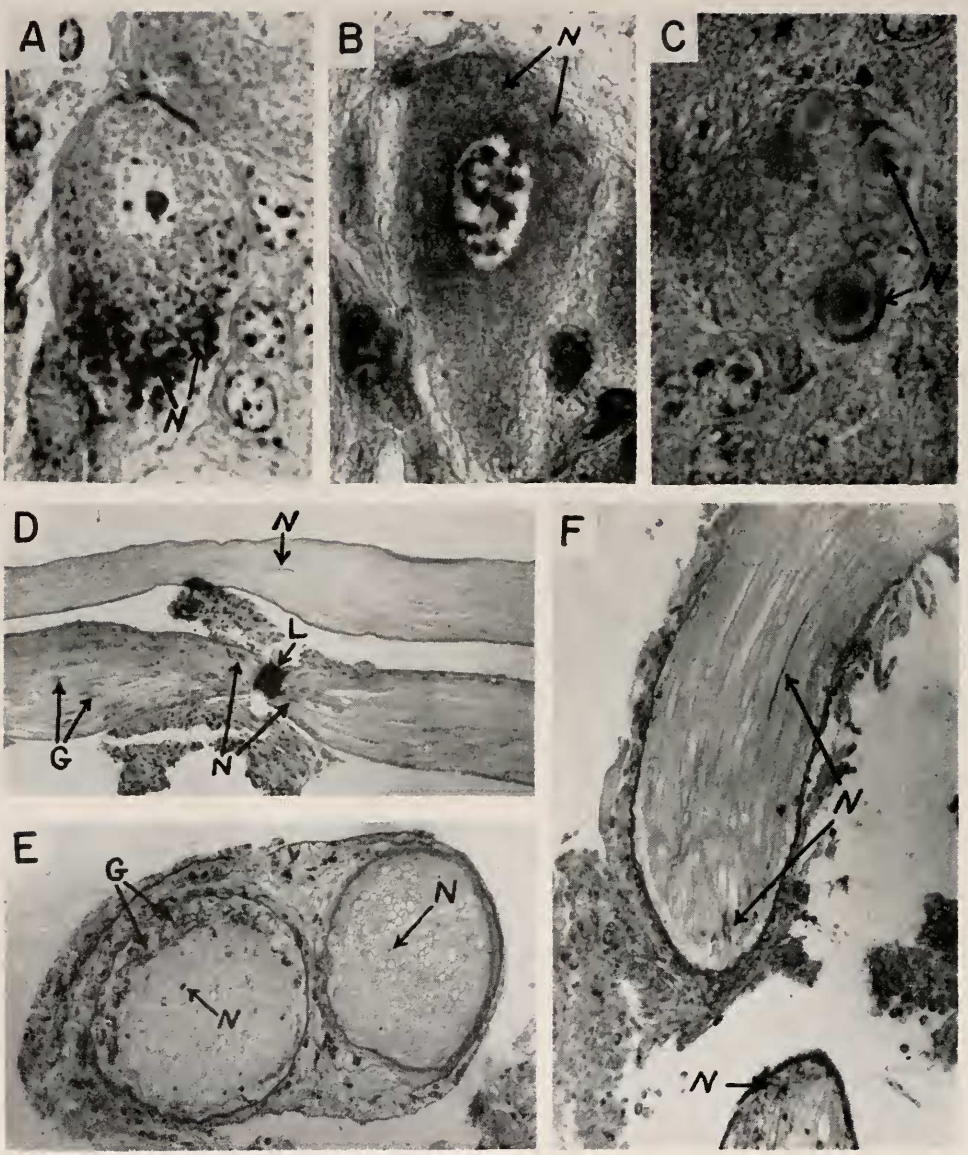

FIGURE 2. Histological preparations. A—type I cell from metathoracic ganglion of adult male (Helly fixation with AF), 1080 ×; B—type II cell from prothoracic ganglion of adult male (Bouin fixation with CHP), 1080 ×; C—type III cell from mesothoracic ganglion of adult female (Helly fixation with AF), 1080 ×; D—frontal section through connective between subesophageal ganglion and prothoracic ganglion (adult male, ligation on animal's right side). Seven days after operation. (Helly fixation with AF), 72 ×; E—cross-section anterior to ligation in left connective between prothoracic and mesothoracic ganglia, control side on right, ♀ adult 20 days after operation (Helly fixation with AF), 144 ×; F—frontal section of left side connective ligated and broken between subesophageal and prothoracic ganglia, ♀ adult, 10 days after operation (Helly with AF). N—neurosecretory materials. G—glia cells. L—ligature.

esophageal ganglion, and connectives of *Periplaneta*. The glia cells are spindle-shaped, have elongated nuclei, and contain gliosomes staining deep purple with AF and deep blue with CHP. In some cases, particularly when the nucleus of the glia cell is not seen, the glia cells might be confused with neurosecretory particles inside connectives, but the latter are in much longer chains and are larger particles than the gliosomes. Roles as supporting cells have been ascribed to the glia cells (B. Scharrer, 1939).

3. *Ligation experiments*

Since ligation of nerves containing neurosecretory axons has been shown to result in accumulations of neurosecretory materials on those sides of the ligations where the neurosecretory materials originate (E. Thomsen, 1954), ligation experiments were used to determine the directions in which the neurosecretory materials were moving. The ligation technique has been described above. In a total of 44 ligation operations, 4 animals died between 10 hours and 12 days after the operation. In 24 cases, the ligated connective was found broken and the nylon thread found in the hemolymph in the vicinity of the operation site. In 16 cases, the connective was not broken and the thread was found still in place (see Fig. 2D).

Whether or not the connective was broken, portions of connective both anterior and posterior to the break or ligation were swollen, as compared to the corresponding unoperated connectives (see Figs. 2D, 2E). The operated connectives, as studied in histological sections, differed from connectives on the control sides in several other ways as well: (a) more glia cells were present on the experimental side, not only in the broken or ligated tips of the connectives, but along their entire lengths (see Fig. 2D); (b) neurosecretory products of types I and II were accumulated in axons on both sides of the breaks, not only localized at the broken or ligated areas, but in elongated masses accumulated within axons on both sides of the operation (Figs. 2D, 2F); (c) no accumulation of type III neurosecretion was seen, doubtless because of its rarity even in the normal connectives.

It is therefore possible to conclude from the ligation experiments that neurosecretory materials from neurosecretory cell types I and II within the thoracic and abdominal ganglia pass in both anterior and posterior directions through the interganglionic connectives. Secretions from type III cells also pass into the connectives but are present in such limited quantities that their movements have not been intercepted in these ligation experiments.

Discussion

With the steadily increasing numbers of neurosecretory cells being described, particularly as new histological procedures are adopted, it is obviously desirable to prevent unnecessary duplication of terminology. Consequently, a more extended comparison of the neurosecretory cells described here with types already described seems appropriate. The most exact resemblance is between the cells which are here designated type II and those designated type B by Nayar (1955) and Köpf (1957). These cells also appear identical to ones staining pink with CHP in the pars intercerebralis of *Blaberus* (Özbas and Hodgson, 1958). Cells of similar staining characteristics have also been seen in the pars intercerebralis of Diptera and Hymenoptera (E. Thomsen, 1954; M. Thomsen, 1954). The uniform dis-

tribution of granules within the cell bodies of all these neurosecretory cells and their staining characteristics strongly suggests that they might properly be considered as homologous neurosecretory cells in several groups of insects.

Type I cells are most nearly comparable with, but not identical to, the cells designated type A by Nayar (1955) and Köpf (1957), and previously seen by other workers in the pars intercerebralis of many insects (Scharrer and Scharrer, 1954; De Lerma, 1956; Dupont-Raabe, 1956; Özbas, 1957). The similarities between type I and type A cells consist of the shapes of the cells and their staining properties with CHP. Nayar describes cytoplasm filled with neurosecretory material in type A cells, but in the preparations of thoracic and abdominal ganglia, the neurosecretory material is always near the edge of the cell body. This arrangement of granules within the cell body resembles that described by Scharrer (1955) in the "castration cells" of the subesophageal ganglia of *Leucophaea*. Nayar noted no selective staining of type A cells with AF. Although the AF stain used here is a later modification of the AF staining technique used by Nayar, typical A cells were seen in the pars intercerebralis of *Blaberus*. Cells of identical staining characteristics, but having cyclic secretory activity, were found in the subesophageal ganglion of *Blaberus* during the present study; these cells have been described earlier by B. Scharrer (1941). The distinction between type I and Type A cells must, therefore, be a real one and not a dissimilarity caused by differences of staining technique.

The type III cells described in this report have not been previously described in any insect. Although the droplets which they contain resemble the "colloid droplets" found in certain neurosecretory cells of *Fundulus* (E. Scharrer, 1941), this resemblance cannot be interpreted as implying identity of cell types in such widely divergent groups of animals.

The conclusions from the ligation experiments are contrary to those drawn from ligation experiments on abdominal cords of *Calliphora* by E. Thomsen (1954). However, the *Calliphora* ligations were performed only as controls for other experiments and the results were not studied histologically. Since few axons in the abdominal connectives carry neurosecretory materials, it is not surprising that Thomsen did not find accumulations of neurosecretions in the ligated abdominal connectives which would be seen by inspection of the whole live connectives. The small quantity of neurosecretory material which would be detected in unligated abdominal connectives probably also explains why none was detected when observations were made upon whole central nerve cords using darkfield illumination (M. Thomsen, 1954).

Attempts to detect effects of neurosecretory products from thoracic and abdominal ganglia upon spontaneous electrical activity of *Blaberus* nerve cords *in vitro*, similar to the effects of neurosecretion from the corpora cardiaca (Özbas and Hodgson, 1958), were unsuccessful. This may be due to the small amounts of neurosecretory materials present even in whole thoracic and abdominal ganglia. Moreover, the most abundant type of neurosecretory cells in these ganglia, type II, is relatively scarce in the pars intercerebralis, and all evidence seems to indicate that it is not their secretion which accounts for the effects of corpus cardiacum extracts upon spontaneous nerve activity (Özbas and Hodgson, 1958).

What may be postulated, then, as a normal function of the neurosecretions

produced in the ganglia and passing anteriorly and posteriorly through the interganglionic connectives? Although the histological sections were always examined with such a possibility in mind, no clues were found in the form of any areas where neurosecretions from the ganglia were normally being accumulated. It must be assumed that the sites of release of the neurosecretory products are widely scattered throughout the central nervous system, regardless of where their effects are ultimately exerted. Concerning this problem of the normal functions of the neurosecretory cells here described, the present observations can really only be an introduction.

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Summary

1. Three types of neurosecretory cells are found within the thoracic abdominal ganglia of the roach *Blaberus craniifer*. These three cell types may be characterized as follows: type I—contains granules staining deep purple with aldehyde fuchsin, the granules being distributed around the periphery of the cell body and in the axon hillock; type II—the most common neurosecretory cell in the ganglia, contains very small granules staining red with chrome hematoxylin and phloxin, the granules being distributed throughout the cell body; type III—the rarest of the neurosecretory cells observed within the ganglia, contains large droplets staining orange with aldehyde fuchsin. The possible identities or homologies of these cell types with others previously described in insects are discussed.

2. Secretory products from all three types of neurosecretory cells have been found within axons in the connectives between ganglia.

3. Ligation of the connectives between ganglia reveals that at least neurosecretory products of cell types I and II move in both anterior and posterior directions from the ganglia in which they are produced. Neurosecretion from cells of type III is very rarely seen in connectives and, consequently, its direction of movement could not be established.

4. No areas of accumulation for any of the neurosecretions were found within the central nerve cords, and the normal functions of these secretions are not known.

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